CLAIMS

1 Novel thrombolytic enzyme named Thrombinase having a molecular weight in the range of 31,000 to 32000 useful for dissolving blood clots

2. Novel thrombolytic enzyme named Thrombinase as claimed in claim1 having a molecular weight of 31700 useful for dissolving blood clots

3.A process for preparation of thrombolytic enzyme, named as Thrombinase having a molecular weight in the of 31000 to 32000 which comprises

(i) Culturing the filtrate of *Bacillus sphaericus serotype* H5a 5b in a culture medium consisting of yeast extract with one or more of constituents selected from peptone, sodium acetate, beef extract, sodium chloride, Soya peptone, and ammonium sulphate

(ii) Removing the cell formed by cross flow filtration using 0.22μ filter,

(iii) Subjecting the cell supernatant thus obtained to two step ultra filtration using 1,00,000 MW (Molecular Weight) cut off membrane followed by ultra filtration of the filtrate thus obtained using 10,000 MW cut off membrane,

(iv) Salting out the retentate with ammonium sulphate,

(v) Subjecting the resulting precipitate to dialysis,

(vi) Re-precipitating the precipitate using ice-cold acetone,

(vii) Reconstituting in buffer,

(viii) Decolorizing by using modified CDR (Cell Debris Remover) treatment, dialyzing, lyophilizing

(ix) Purifying firstly by ion exchange chromatography followed by gel filtration chromatography and

(x) Dialyzing the fraction showing fibrinolytic activity and lyophilizing to obtain purified Thrombinase having a molecular weight in the range of 31,000 to 32000 Daltons.

4. A process as claimed in claim 3 wherein the amount of the constituents present in the Culture medium employed is 0.03 to 1.5% of yeast extract, 0.2 to 1.5% peptone, 1 to 1.6% sodium acetate, 0.3 to 0.5% beef extract, 0.2 to 0.5% sodium chloride, 0.5 to 1% Soya peptone, and 0.68% ammonium sulphate

5. A process as claimed in claims 3 & 4 wherein the pH of the culture medium used is in the range of 7.2 to 8.0.

6. A process as claimed in claims 3 to 5 wherein the amount of ammonium sulphate used is in the range of 20 to 40%.

7. A process as claimed in claims 3 to 6 wherein the buffer used is Tris 0.01 M and the pH is 8.0.

8. A process as claimed in claims 3 to 7 wherein the amount of ice-cold acetone and crude enzyme used are in the ratio of 1:1 to 1:1.5 (v/v).

9. The use of the novel enzyme named Thrombinase having a molecular weight in the range of 31,000 to 32000 for dissolving blood clots.